

## Analyzing the Dynamics of Goat Milk Clotting with Animal and Microbial Enzymes

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### ABSTRACT

This article provides the results of studying the clotting of scald goat milk with animal and microbial enzymic preparations. The study was aimed to determine the optimal enzymic preparation dose for coagulation, considering the quality of a milk curd at various enzyme doses. The enzymic preparation coagulation rate was evaluated by the test instrument technique, whereas the milk coagulation rate was evaluated on instruments that allowed registering in-coagulation structural changes in the milk formula and determine the strength of milk curds. The studies allowed registering the results of the milk-clotting activity of the animal and microbial enzymic preparations in goat milk on an instrument that recorded the clotting rate and duration. The optimal milk-clotting activity is one of the main properties affecting the curd quality in the production of cheese. The test unit was also used to check the curd strength by immersing an indenter after the coagulation using the animal and the microbial enzymic preparation.

Thus the results allow adequately determining the optimal enzyme dosage and the duration of clotting. According to the studies, the microbial milk-clotting enzyme had a higher clotting rate but yielded weaker curds as compared with their animal counterparts.

### KEYWORDS

Enzyme, Goat Milk, Coagulation Rate, Curd Strength, Calcium Chloride, Halloumi.

### Introduction

The current range of products made from goat milk is not very broad. Goat milk has been accepted as a raw material only partially [1].

The main difficulty faced by the technologists in the production of cheese is the peculiar features of goat milk protein coagulation.

Milk-clotting enzymes are the key link in the milk clotting process. Milk is a liquid with a lot of kinetic separates. With the addition of an enzyme, however, it passes from one state to another

[2].

When assessing the suitability of milk-clotting preparations for the production of cheese, it is necessary to take into account their milk-clotting activity. The optimal milk-clotting activity is one of the main characteristics affecting the clot quality in the production of cheese[3].

According to the specialized literature, cheese-making enterprises are offered a broad range of both, animal and vegetational milk-clotting enzymes [4].

Milk-clotting enzymic preparations can be tentatively divided in enzyme rennets and their substitutes. The latter include animal, vegetational, and microbial substitutes as well as recombinant chymosin [5 and 6].

The two main milk-clotting enzymes included in enzyme rennets are chymosin and pepsin. Several comparative studies of the properties of animal enzymes and enzymic substitutes of enzyme rennets are considered in works by such researchers, as I. Rogelj, B. Perko, A. Francky, V. Penca, J. Pungerčar[7 and 8].

The scarcity of enzyme rennet has made it necessary to search for new alternative sources of milk-clotting enzymes. In addition, enzyme rennet substitutes had to meet a number of cheese-making requirements the compliance with which would result in high-quality products [9].

The advantage of microbial and vegetational enzymic preparations is their low prime cost [10 and 11].

It is known that milk clotting phases can be studied at both levels, commercial and laboratory, by several methods described in works by D. J. O'Callaghan, C.P. O'Donnell, F.A. Payne, D.J. Mc Mahon, and R.J. Brown. The proposed devices are based on mechanical, vibrational, and optic properties. The mechanical ones make use of the resistance produced by the clot; the vibrational ones make use of probes that produce different vibrations in response to milk clotting; the optic devices register changes in the properties, colour, and absorption of milk during clotting phases[12 and 13].

Thus this work aims to examine the milk-clotting activity of animal and vegetational enzymic preparations in goat milk and determine the optimal preparation dose for the production of halloumi cheese [14 and 15].

## **Materials and Methods**

The experimental studies were conducted in Barnaul at the laboratories of the Federal State Scientific Institution Siberian Research Institute of Cheesemaking, Federal Altai Scientific Center for Agrobiotechnology (SibNIIS) and repeated five times.

The enzymic preparations chosen to determine their milk-clotting activity for making halloumi goat milk cheese were animal preparation SG-50 and Renin made by microbial synthesis.

The SG-50 Normal rennet beef enzyme is a natural powdery preparation containing chymosin and

beef pepsin at a ratio of 50:50. It is known that the cheese-making tests with the 50/50 chemosin-beef mix were especially successful.

The Renin microbial enzyme is a next-gen enzyme made from the recombinant chemosin fermented with the *Mucor miehei* strain. Renin is of high microbial purity, exhibits high proteolytic action, and helps improve syneresis; as a result, the whey is easily yielded by the grain. Moreover, Renin has a marked splitting effect on kappa casein, which preconditions good clustering, and favours a higher output of ready-made product and the correct formation of its organoleptic properties (flavour and texture).

To adequately evaluate the milk clotting process, the coagulation dynamics was analyzed using a special unit based on a MPU developed in the SibNIIS by Professor A. A. Mayorov [16].

The instrument was intended for making nondestructive measurements of rheological properties of rennet curds. The unit was designed as a mechanical system for swinging a small cup pre-filled with milk and the enzyme preparation. The strong point of this method is that it allows nondestructively examining various milk curd samples. The instrument's operating principle is based on fixing the deviation of a laser beam reflected from the surface of the examined milk formula. The beam reflected from the examined sample surface falls on a light detector. In the first milk clotting phase, with the change in the tilt of the cylinder with the product sample, the position of the beam on the screen will not change. When the structure is formed or the viscosity changes, the position of the cylinder relative to the horizon changes at tilting. The change in the beam's reflection is measured in mV using a special light detector [17].

To study the milk clotting dynamics on the test unit, several tests were conducted with different doses of the enzyme and concentrations of the applied doses of calcium chloride [18]. The milk-clotting enzymes used were rennet enzyme SG-50 and microbial enzymic preparation «Renin».

The experiment was made on the goat milk taken at the Pilot Cheesemaking Plant (OOO ESZ) supplied there from a farming enterprise in Barnaul. The milk was pasteurized for 20-25 seconds at  $T = 71 \pm 1$  °C.

The actual dose of the inoculum was determined by varying its fraction from 1.5 to 3 %. The fundamental condition for the experiment was that the fermentation and the formation of a dense curd should take 20 to 30 minutes for intensifying the production activity and obtaining a good-quality milk curd.

The procedure of the test on the pilot unit is exposed below. First, a milk sample of 100 ml was heated to  $T = 32$  to  $34$  °C and the rennet was added. Then the milk was immediately poured to a cylindrical cup of 100 ml. The cup was closed with a cover to maintain the preset temperature; then, it was fastened to the unit's mobile frame. The data about the position of the laser beam reflected from the tested sample surface were recorded every 20 seconds at the moment, when the cylinder was tilted.

The property studied in parallel to studying the clotting dynamics was the limit strength of the rennet curd; in its respect, the latter is the curd quality indicator.

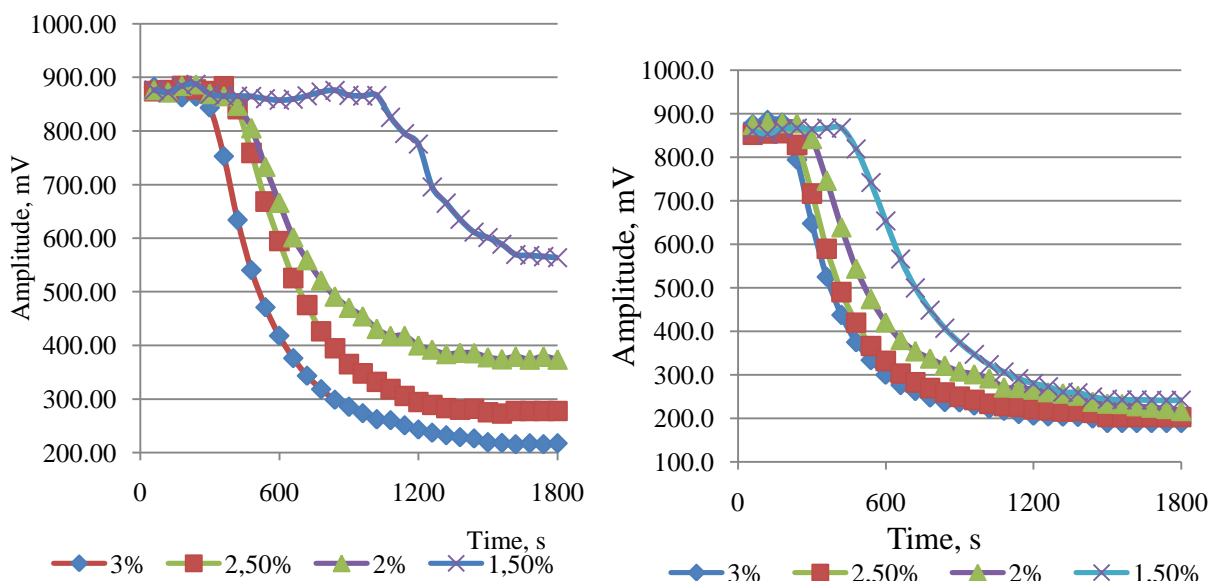
The instrument's operating principle is based on measuring the rennet curd strength limit by immersing an indenter into a fermented dairy medium. The measured results are converted to grams

and displayed on the monitor as well as recorded in the memory of the computer. Thus this method of studying rennet clotting is based on measuring the resistance caused by the indenter immersed in the rennet curd.

## Results

The obtained data were used to build the plot shown in Figure 1. The curves in this figure exhibit different amplitudes of the deviation of the laser beam, reflected from the milk surface, in the course of structural changes in the milk up until the formation of a curd.

This process can be characterized as follows: with an increase in the enzymic preparation concentration, the degree, to which the laser beam is reflected from the rennet curd surface, goes down, which is confirmed by the reduction in the beam reflection frequency characterizing the dynamics of clotting. The analysis of the results allows concluding that the test unit makes it possible to track the main sections, time, and rate of rennet clotting.



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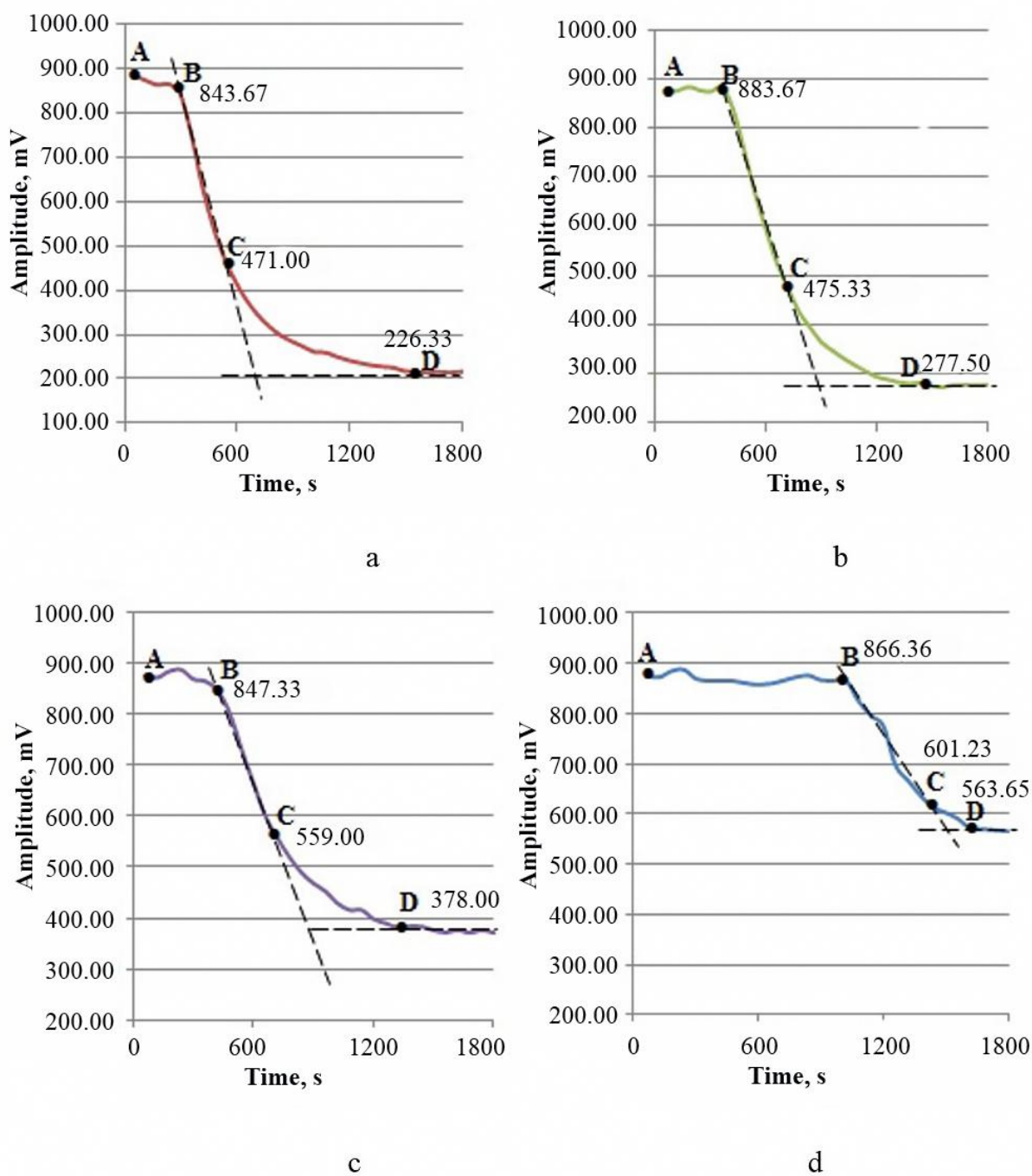
a is EP SG-50; b is EP «Renin»

**Figure 1.** Fermentation time dependence of the amplitude of the beam reflection from the surface of the enzymed goat milk mix

Note that at small applied doses of enzymic preparations it is seen that the starting phase of clotting, that is, the removal of glycomacropetide, was attended by the formation of a certain structure; however, it was weak and got destroyed in the subsequent tests (curd strength limit measurements).

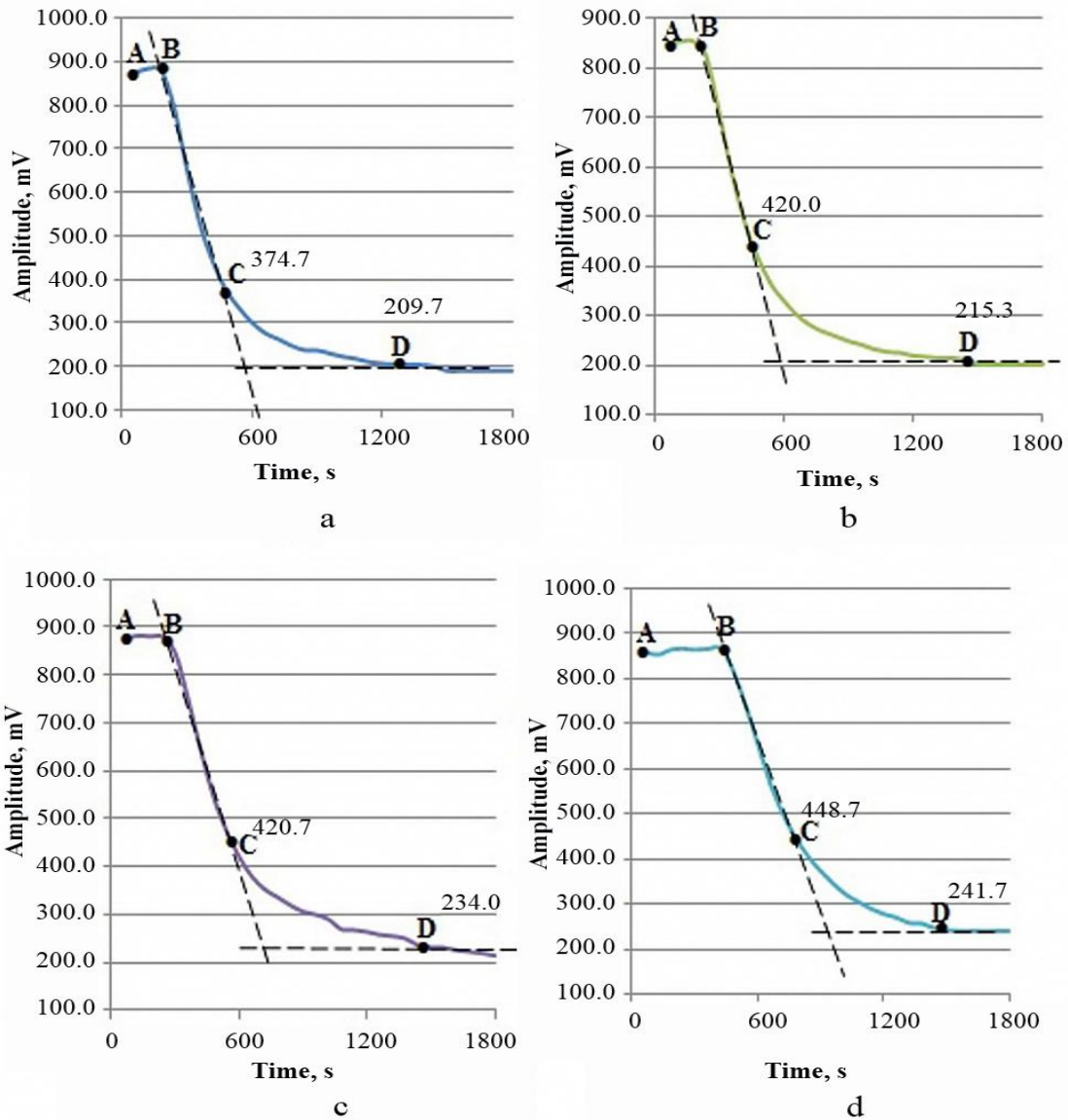
To study the main phases of goat milk structuring, the clotting curves were exposed to graphical differentiation.

For the main four sections of goat milk clotting that were determined by graphical differentiation see Figures 2 and 3.



a is 3 % ; b is 2.5 % ; c is 2 % ; d is 1.5 %

**Figure 2.** Graphical differentiation of the dependence of goat milk clotting on the dose of EP SG-50



a is 3 % ; b is 2.5 % ; c is 2 % ; d is 1.5 %

**Figure 3.** Graphical differentiation of the dependence of goat milk clotting on the dose of EP «Renin»

According to the exposed curves, section A-B is for the preventive phase. In this phase the milk is prepared for clotting, that is, glycomacropetide is removed. According to the curves shown in Figures 2 and 3, this process varies in duration and changes, depending on the composition of milk and the enzyme concentration. Section B - C is for the initial structuring phase and characterized by a linear structuring rate; the next section is C - D showing the slowdown in the structuring rate; the final section is D in which the structure is strengthened and the release of whey begins.

The tangent rule was used to identify points A, B, C, and D. The active structuring period extended from point B to point C. The latter is the point of separation from the constant rate on

the curve to the dropping point D, and the one from which the structuring rate smoothly goes down. D is the point of separation from the dropping rate and the beginning of the structuring process finish.

In this case, the first derived function is equal to the tangent of the angle between the X-axis and the tangent line drawn to the curve in the derivative calculation point:

$$dA/d\tau = \text{tg}\alpha(1)$$

where  $dA$  is the amplitude in mV;  $d\tau$  is the goat milk clotting time in minutes.

As seen from analyzing the exposed plots, the value of the final point of the initial structuring phase goes up depending on a reduction in the enzyme dose and the degree, to which the beam is reflected from the fermented medium surface, goes up accordingly.

According to the regularity discovered by comparing separation point C and final structuring point D, that were found by the tangent method, the beam reflection amplitude value goes up with a reduction in the EP dose, which is due to the structural changes in the milk clotting process.

The linear values of the B-C sections are characterized by a dropping curve and can be used for calculating the goat milk clotting rate.

The parameters calculated for the purpose were the change in the amplitude between B and C and the respective time between such for the axis of time. The quotient of the difference in the amplitude values and the difference in time in B and C will be the clotting rate.

The goat milk clotting rate was found by formula (2) as

$$v = (A_B - A_C) / (\tau_B - \tau_C) \quad (2)$$

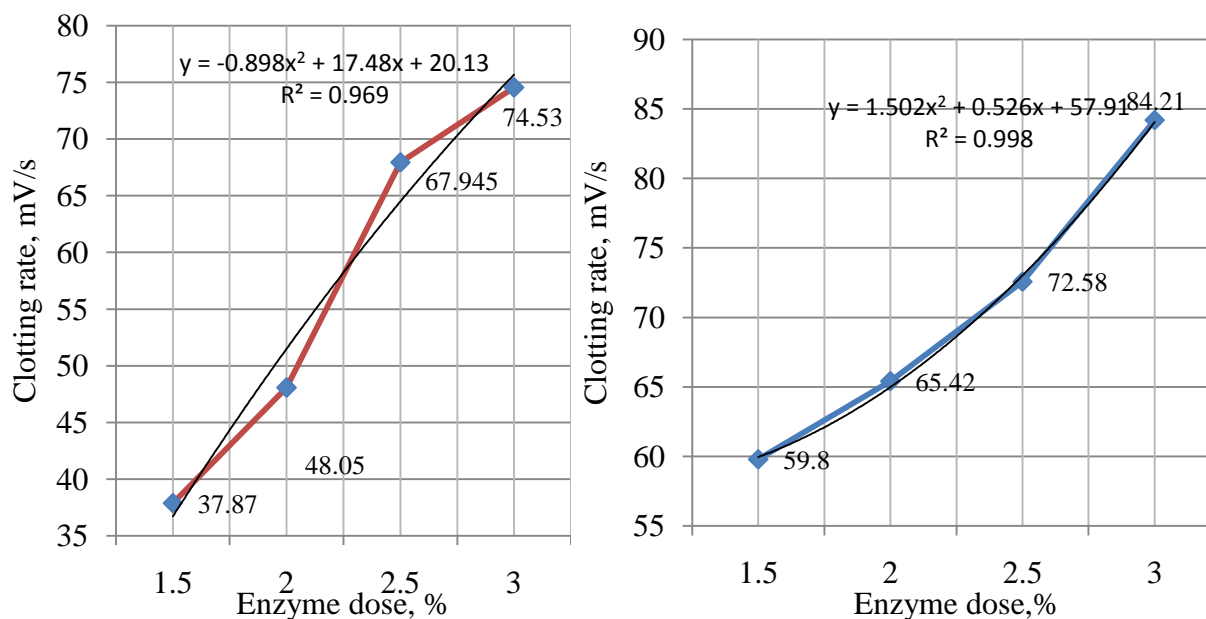
Where  $A_B$  is the amplitude in point B, mV.

$A_C$  is the amplitude in point C, mV.

$\tau_B$  is the time corresponding to point B, minutes.

$\tau_C$  is the time corresponding to point C, minutes.

In the next phase of the goat milk clotting test the plots were drawn that reflected the clotting rate corresponding to the weakening in the amplitude of the beam reflected from the enzymic mix surface. See Figure 4 for the dependence between the goat milk clotting rate and the enzymic preparation dose.



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a is EP SG-50; b is EP «Renin»

**Figure 4.** Dependence of the goat milk clotting rate on the dose of EP SG-50 and «Renin»  
 While analyzing the plots shown in Figure4, it becomes clear that the clotting rate attained using Renin is by 11 to 36 % on average than the rate attained using SG-50.

This process is described by a linear equation with an approximation close to 1 (Table 1), which validates the reliability of describing the maximal clotting rate, depending on the applied dose of EP.

**Table 1.** Summarized data from the mathematical model of the influence of the enzymic preparation dose on the maximal clotting rate

EP	Equation	Determination ratio	Correlation ratio
SG-50	$Y = -0.8987x^2 + 17.481x + 20.136$	0.9696	0.9846
Renin	$Y = 1.5025x^2 + 0.5265x + 57.918$	0.9987	0.9993

Since the correlation ratio of the developed mathematical models is close to 1, it can be concluded that they adequately describe the influence of the dose of EP on goat milk clotting.

Then the results of measuring the strength of the milk curds obtained by the coagulation using SG-50 and Renin were studied. For the results of those studies see Table2.

**Table 2.** Curd strength limits resulting from the coagulation of goat milk by means of SG-50 and Renin

Enzyme dose, %	Curd strength limit	
	SG 50	Renin
1.5	18.63	13.9
2	19.06	18.82



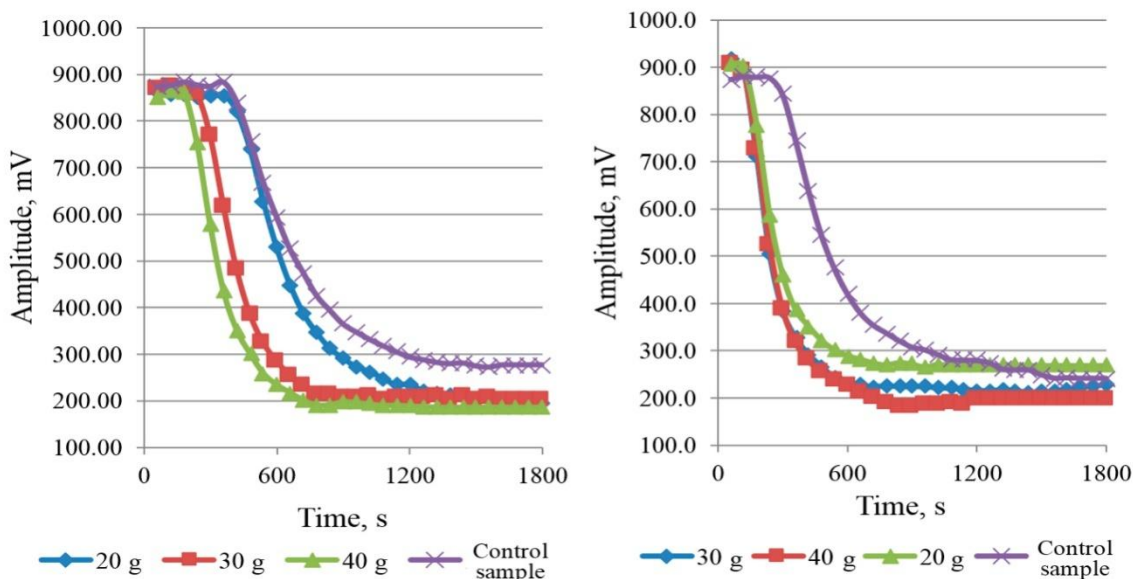
2.5	20.79	19.71
3	22.38	20.91

As shown by analyzing the data, the curds resulting from the coagulation of goat milk by means of SG-50 resisted a greater load of the indenter in comparison with the curds resulting from the coagulation of goat milk by means of Renin. It might be connected with the fact that the coagulation by means of the animal enzyme was a more stable process attended by an even separation of whey. At the same time, the clotting at the coagulation by means of Renin was more intensive due to its high proteolytic activity and attended by an uneven separation of whey, which then affected the rheological properties of the ready-made curd.

Thus the first part of the experimental studies of the dependence of the goat milk curd strength on the enzymic preparation dose and the clotting rate allowed choosing the optimal enzymic preparation dose of 2.5 %, which is also confirmed by the economic expediency of using enzymic preparations that results in good-quality clots.

It should be particularly emphasized that, in the course of milk curd structuring an increase in the calcium content and the connection of calcium with the other components of milk play an important role in cluster formation. Active calcium is released from colloidal phosphate nanocluster progressively as the pH in the casein micelli goes down.

The next phase of the studies was the determination of a sure application dose of calcium chloride for pasteurization changes the protein fraction of milk, that is, it lowers the soluble protein content and downgrades the salt composition of calcium due to sedimentation [18]. The latter plays a major role in curd formation, which is confirmed by the works of several Russian and foreign researchers, such as Irina Mikhailovna Mironenko, K. Sievanen Huppertz, G. Singh, S. Arora, G. S. Sharma, J. S. Sindhu et al. [19, 20, 21, 22]. The salt balance recovery implies adding calcium chloride to milk. The coagulation of goat milk was studied upon the application of 40, 30, and 20 g of calcium chloride. See Figure 5 for the results of those studies.

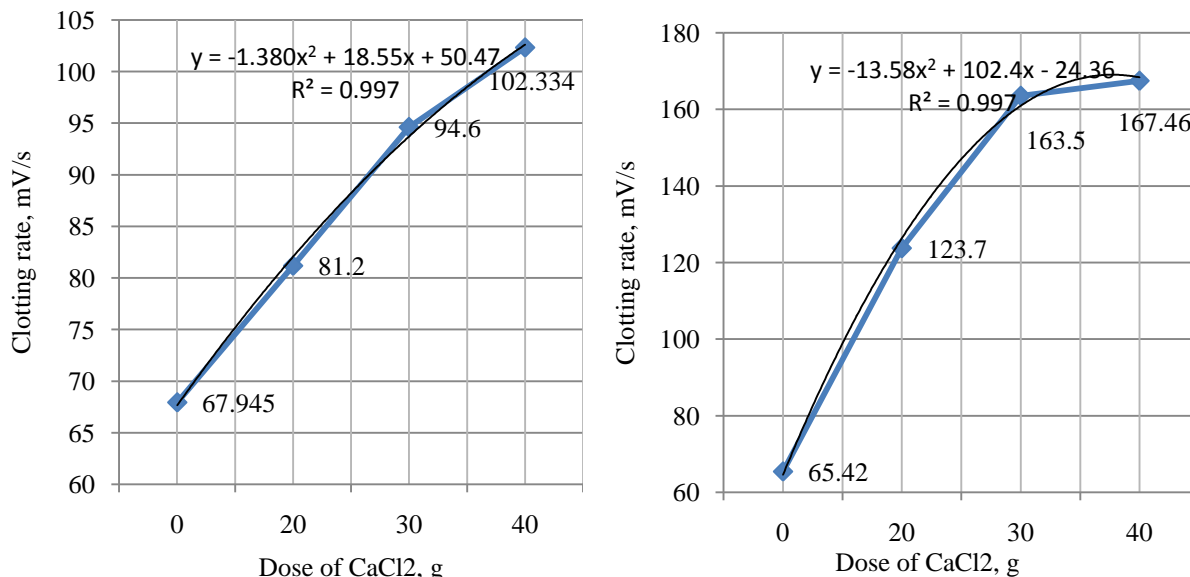


a is EP SG-50; b is EP «Renin»

**Figure 5.** Fermentation time dependence of the amplitude of the beam reflection from the surface of the fermented goat mix at various CaCl<sub>2</sub> application doses

The addition of calcium chloride accelerated clotting by 33-50%.

Then the changes in the clotting rate as a result of the coagulation using SG-50 and Renin on linear sections B and C were studied. See Figure 6 for the dependences of the goat milk clotting on the dose of calcium chloride.



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a is EP SG-50; b is EP «Renin»

**Figure 6.** Dependence of the goat milk clotting rate on the concentration of CaCl<sub>2</sub>

The results show that the application of CaCl<sub>2</sub> favours a more intensive clotting process. Thus, with an increased in the dose of calcium chloride, the clotting rate would significantly rise as compared with the control sample. With the application of the maximal dose of calcium chloride, the clotting rate rose by twofold when both, SG-50 and Renin, were used.

See Table 3 for the combined mathematical models of the maximal goat milk clotting rate and the curd's readiness for cutting, depending on the applied dose of calcium chloride.

**Table 3.** Mathematical models of the clotting rate and the curd's readiness for cutting at the coagulation of goat milk by means of SG-50 and Renin

EP	Equation	Determination ratio	Correlation ratio
Clotting rate			
SG-50	$Y = -1.3803x^2 + 18.558x + 50.477$	0.9975	0.9987
Renin	$Y = -13.58x^2 + 102.49x - 24.36$	0,9978	0.9988
Curd's readiness for cutting			
SG-50	$Y = -0.25x^2 - 1.25x + 19.75$	0.9618	0.9807
Renin	$Y = 3.75x^2 - 17.05x + 25.75$	0.9993	0.999

According to this table, the developed models have a determination ratio close to 1, which allows concluding that these mathematical models adequately describe the dependence between the influence of the calcium chloride dose on the clotting rate and the curd's readiness for cutting.

See Table 4 for the curd strength limits attained by the coagulation using SG-50 and Renin, depending on the applied dose of calcium chloride.

**Table 4.** Curd strength limits resulting from the coagulation using SG-50 and Renin

Applied dose of CaCl <sub>2</sub> , g/100 kg	Curd strength limit, kPa	
	SG-50	Renin
20	22.18	19.43
30	24.46	20.48
40	29.95	20.63
Control sample	20.79	18.82

As confirmed by Table 4, the application of calcium chloride favoured the formation of harder curds and increased their strength by 9 to 31 %. It should be noted that the curd strength limits attained by the coagulation using SG-50 were higher than those attained using Renin.

## Discussion

According to the results of studying the influence of calcium on milk clotting, the amount of calcium at a constant dose of enzyme can significantly intensify milk clotting and thus allow producing good-quality milk curds.

As seen from analyzing the goat milk curd strength, attained by adding Renin, it is lower than the curd strength attained using SG-50. The goat milk curd strength attained by the coagulation using SG-50 is by 9 to 31 % higher on average than the one attained by the coagulation using Renin. It should be noted that the curd strength affects the milk components's binding degree at coagulation, which allows avoiding the loss of dry substance on whey.

## Conclusion

Thus the investigational studies have allowed exploring the milk-clotting activity of animal and microbial enzymic preparations in goat milk. It has been established that the more intensive milk-clotting activity is shown by the Renin microbial synthesis preparation and that the milk curd obtained using the animal enzymic preparation has a better quality.

The study results can be used in the research and production activities aimed at making semihard goat milk cheese. In addition, the results will help grasp the essence and evaluate the milk-clotting action pattern of microbial enzymic preparations that are currently less well understood.

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<http://annalsofrscb.ro>

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